Project meeting
„Joint chemical laboratory for the service of bioeconomy in the Slovak-Hungarian border region"

# Bioethanol Production from Renewable Raw Materials

**Interreg, SKHU/1902/4.1/001/Bioeconomy**

**Slovak University of Technology in Bratislava**

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B. Horváth[a], R. Barthos[b], A. Kaszonyi[a]

*[a] Slovak University of Technology in Bratislava, Faculty of Chemical and Food Technology, Bratislava, Slovakia*
*[b] Eötvös Loránd Research Network, Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry, Budapest, Hungary*

# Biomass and its uses

In most developed countries, governments stimulate the use of renewable energies and resources with following major goals:

- (*i*) to secure access to energy,
- (*ii*) to mitigate climate changes,
- (*iii*) to develop/maintain agricultural activities and
- (*iv*) to ensure food safety.

During the last decades of the 20th century, there was an enormous interest in the production and usage of **liquid biofuels** (biodiesel or bioethanol) as promising substitutes for fossil fuels.

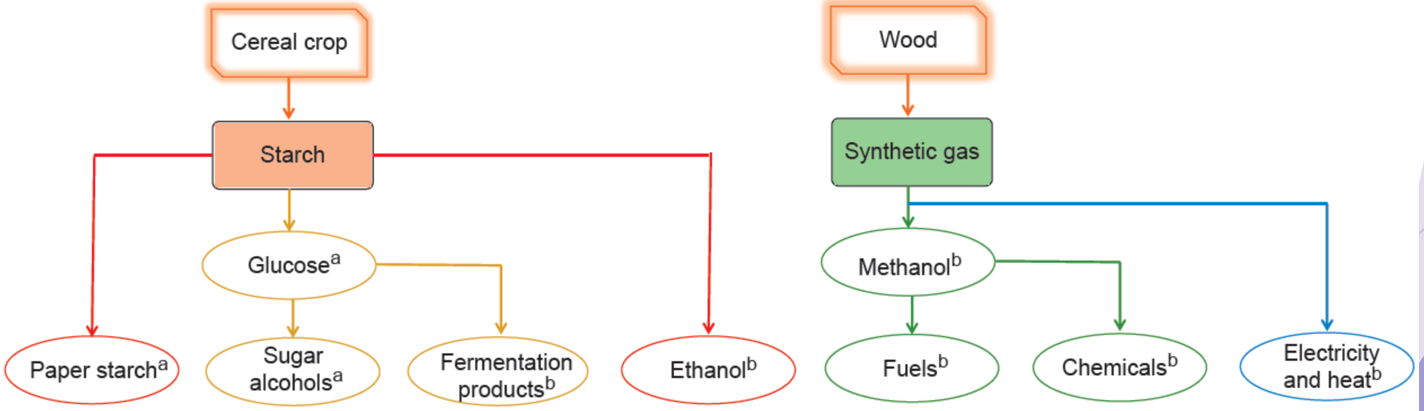
Bioethanol is typically produced via **microbial fermentation** of fermentable sugars, such as glucose, to ethanol.

# Bottom-up approach:

An example of bottom-up biorefinery is the wheat and corn starch biorefinery that starts as a simple starch factory -Lestrem, France, USA (Decatur, Illinois), Austria (Lenzing) and Norway (Sarpsborg).

# Top-down approach:

The new top-down approach is a highly integrated system established for the use of various biomass fractions and generation of different products for the market (Green Biorefinery Upper Austria – green grass juice, utilization of N, P, *Wautersia eutropha*)



Cereal crop
Starch
Glucose[a]
Paper starch[a]
Sugar alcohols[a]
Fermentation products[b]
Ethanol[b]

Wood
Synthetic gas
Methanol[b]
Fuels[b]
Chemicals[b]
Electricity and heat[b]

a-traditional, b-new products

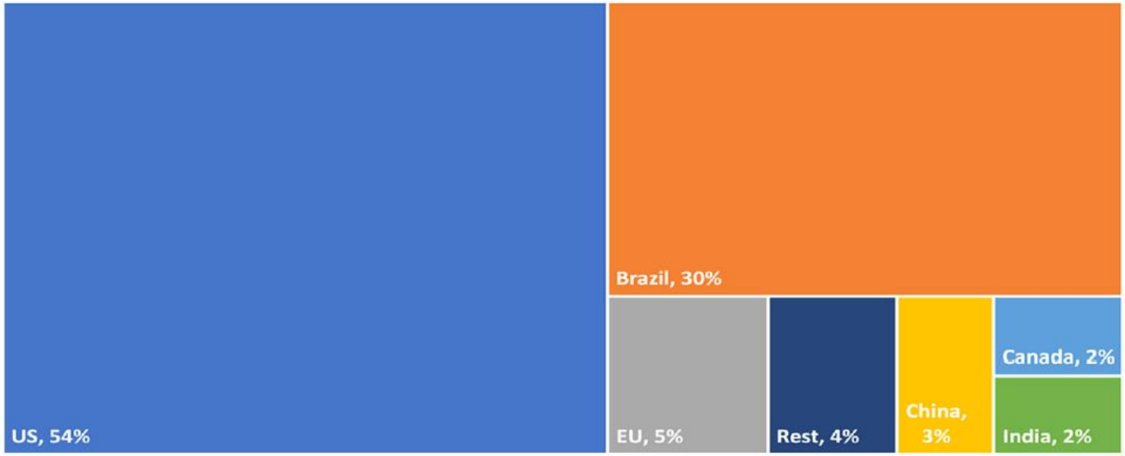
# Sources of bioethanol

In Europe, in 2019 just 4.3% of the total bioethanol produced came from **nonedible** lignocellulosic biomass. In 2019 the European renewable ethanol installed production capacity was 9.9 Mm$^3$, while the ethanol imports in Europe reached 1.3 Mm$^3$.

- 48.6% of the renewable ethanol produced in Europe was from corn
- 21.1% wheat
- 19.3% sugar
- 6.7% cereals, crops, starch
- 4.3% lignocellulosic and others

- The current global economy is based on **linear** economy, which consists on production-consumption-dispose.
- C**ircular** economy would consist on a closed loop of production-consumption-recovery-production.
- It is estimated that just 9.1% of current global economy is circular (2020).

# Ethanol production and cost



106 $Mm^3$ –2016
130 $Mm^3$ -2024

In 2019, projected portion of US in 2024: 42%

Nearly 40% from sugar cane and beet
Nearly 60% from starch-containing

Sugar cane, Brasil – 0.20-0.30 USD/l
Sugar beet, corn, EU, US – 0.30-0.53 USD/l
Wheat, sweet potato, China – 0.28-0.46 USD/l
Simple sugars, India – 0.44 USD/l
Lignocellulose, average – 0.80-1.20 USD/l
Gasoline, refining cost – 0.10-0.18 USD/l (2018)

# Raw materials containing sugar

- 2/3 of the world sugar production are from sugar cane
- 1/3 from sugar beet
- Sugar cane semi-perennial, less agricultural operation
- Molasses: 50-60 % sugar (starch and citrus molasses 40-45%)
- Whey as a by-product in cheesemaking (4.9% lactose)

# Raw materials containing starch

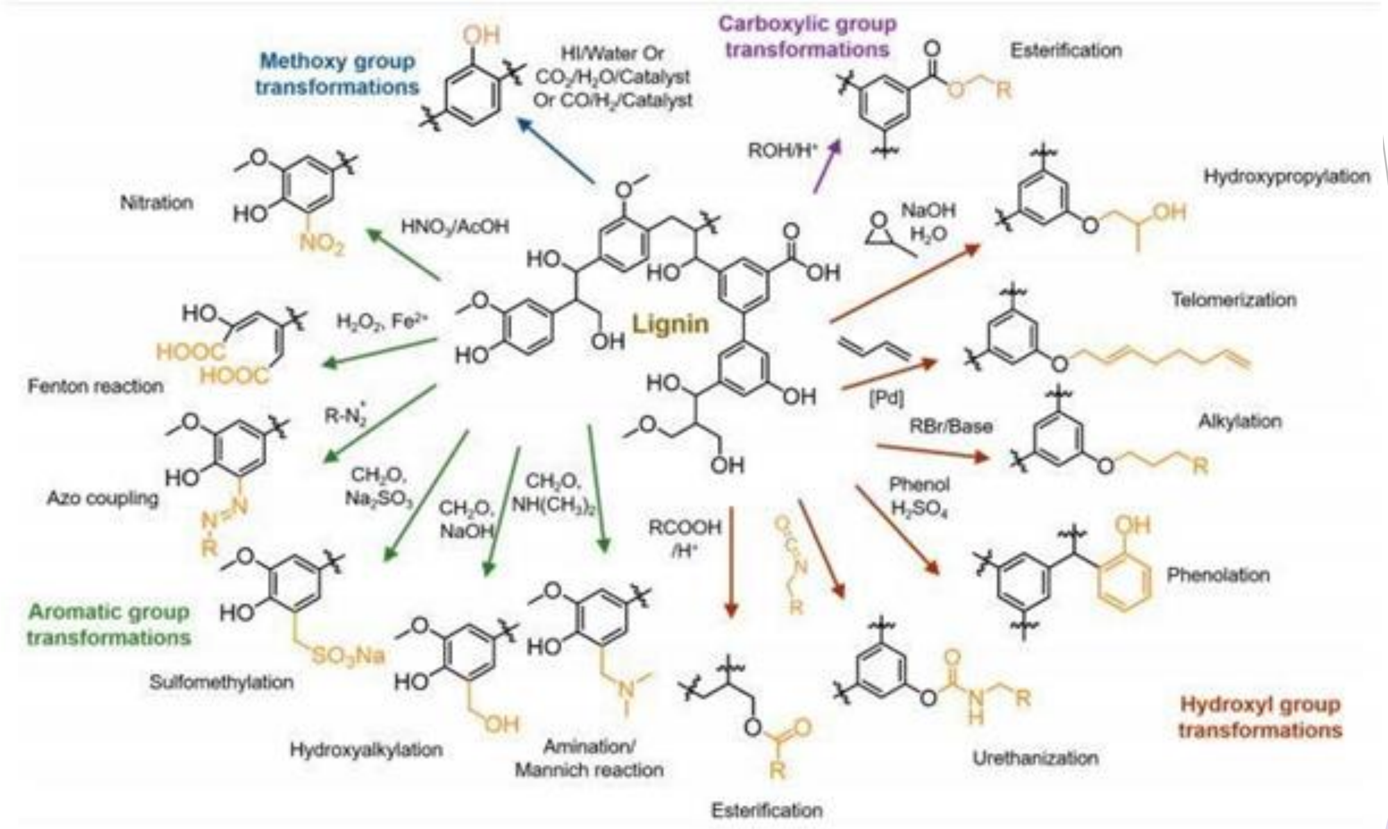
- USA represents 80% of the worldwide starch market (95% of bioethanol from corn)
- Hydrolysis by amylase, glucoamylase
- *Bacillus licheniformis* and modified  produces amylases
- Max. efficiency 51% by mass, in practice 40-48%
- Microalgae convert $CO_2$ to lipids and sacharides

# Raw materials containing lignocellulose

- Renewables non-competitive with food crops
- More evenly distributed, cheaper than sugars, arduous pre-processing
- Crop residues (straws, stovers), wood, cellulose wastes (paper), grasses, municipal waste
- Pretreatment steps necessary:
  - Alkaline (NaOH etc.)
  - Acids ($H_3$PO4, $H_2SO_4$ etc.)
  - Organosolv (EtOH, glycerol at cca. 200 °C)
  - Ionic liquids
  - Biochemical (*Ceriporiosis subvermispora*, 18 days)

- Formation of toxic compounds in harsh conditions (furans, phenolic compounds, ketones) can be subsequently removed:
  - By extraction
  - Adsorption
  - Enzymatically
  - By using resistant strains

# Utilization of lignin



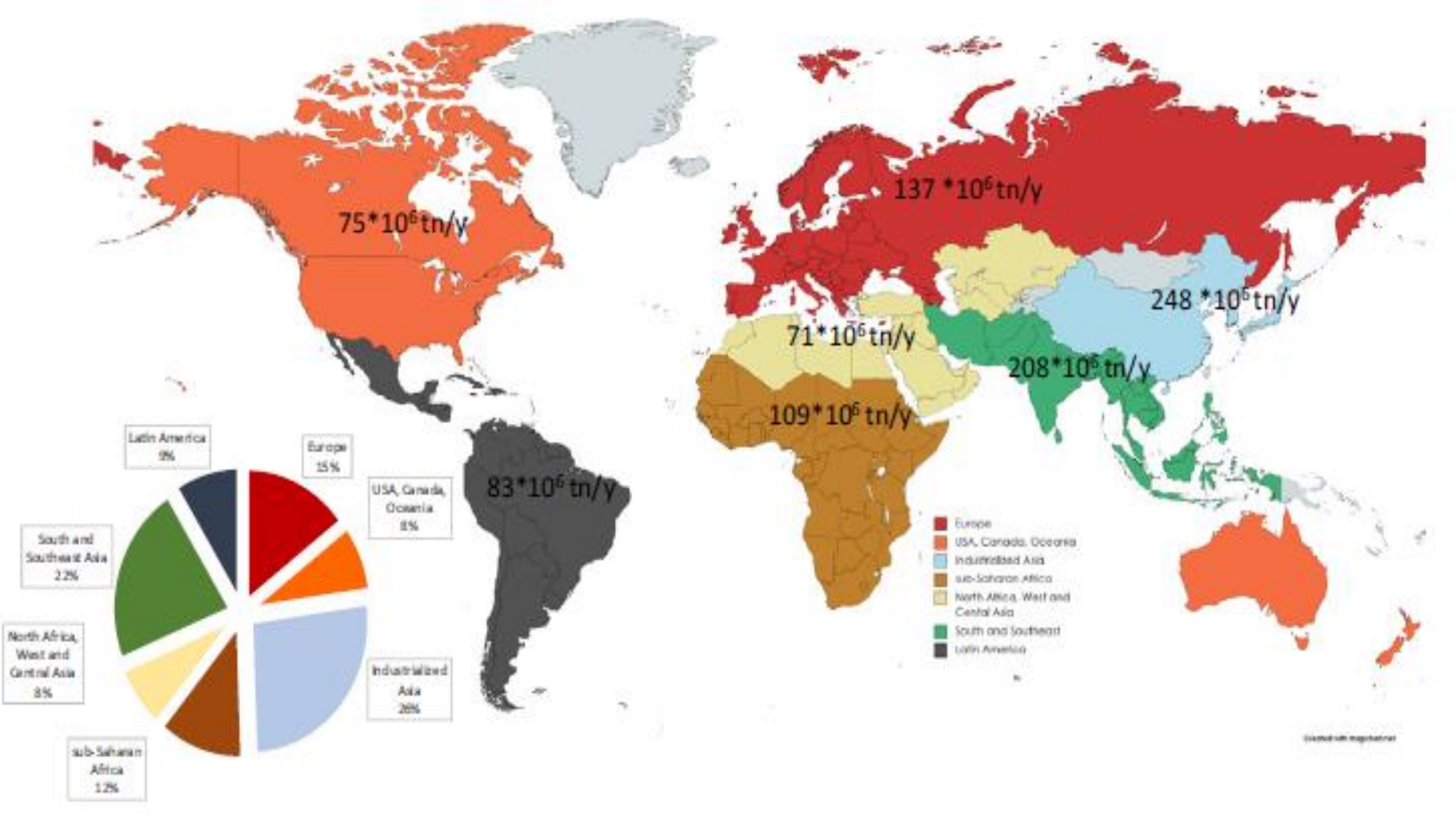
Souza RE, Gomes FJB, Brito EO, et al. A review on lignin sources and uses. *J Appl Biotechnol Bioeng*. 2020;7(3):100-105.

The biochemical transformations of lignine are of great interest.
Inhibitive effects: formation of formaldehyde from -OMe groups

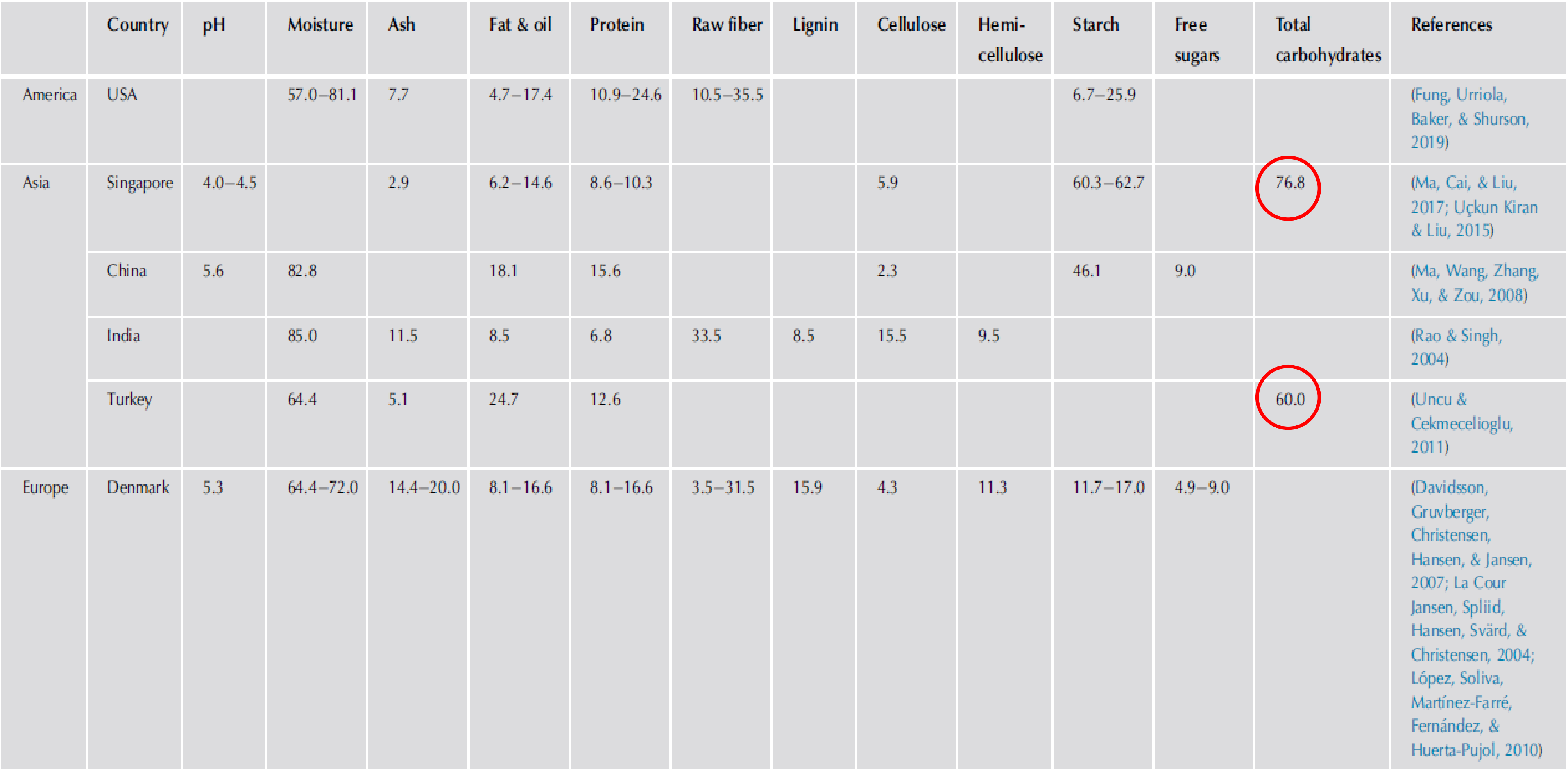
# Reported pretreatment methods

| Feedstock | Pretreatment/ hydrolysis | Microorganism | $\gamma$(ethanol) / g/L | $E$ / % | $P_{max}$ / g/(L·h) |
|---|---|---|---|---|---|
| Sugar cane bagasse | Diluted acid ($H_2SO_4$) followed by alkaline delignification (NaOH), cellulase complex obtained from *Trichoderma reesei* (MULTIFECT®) | Recombinant *Saccharomyces cerevisiae* containing the β-glucosidase gene from *Humicola grisea* | 51.7 | – | 0.94 |
| Bagasse | Acid ($H_3PO_4$), Accellerase® 1500 enzyme | *Z. mobilis* ATCC 29191 immobilized in Ca-alginate (CA) and polyvinyl alcohol (PVA) gel beads | 6.24<br>5.52<br>5.53<br>5.44 | 79.09<br>69.96<br>70.09<br>68.95 | 3.04<br>2.37<br>1.31<br>1.27 |
| *Eucalyptus globulus* wood | Organosolv (50 % EtOH, 200 °C, 45 min), cellulase (Celluclast) and β-glucosidase (Novozym 188) | *S. cerevisiae* IR2T9-a | ~42 | – | – |
| Rice straw | Alkali (NaOH), Accellerase® 1500 enzyme | *S. cerevisiae*, *Candida tropicalis*, *S. stipitis* | 28.6 | 86 | – |
| Corn stover | AFEX commercial enzymes mixture (Ctec 2, Htec 2 and Multifect pectinase) | Genetically engineered *S. cerevisiae* Y35 | 45.5<br>51.3 | –<br>– | 0.87<br>0.76 |
| Cellulosic material, β-glucan | - | Recombinant *Kluyveromyces marxianus* K1 | 4.24 | 92.2 | 0.55 |
| Corn stover | AFEX | *Clostridium phytofermentans* (ATCC 700394) | 7.0 | – | – |

# Ethanol from food waste
## Food waste production

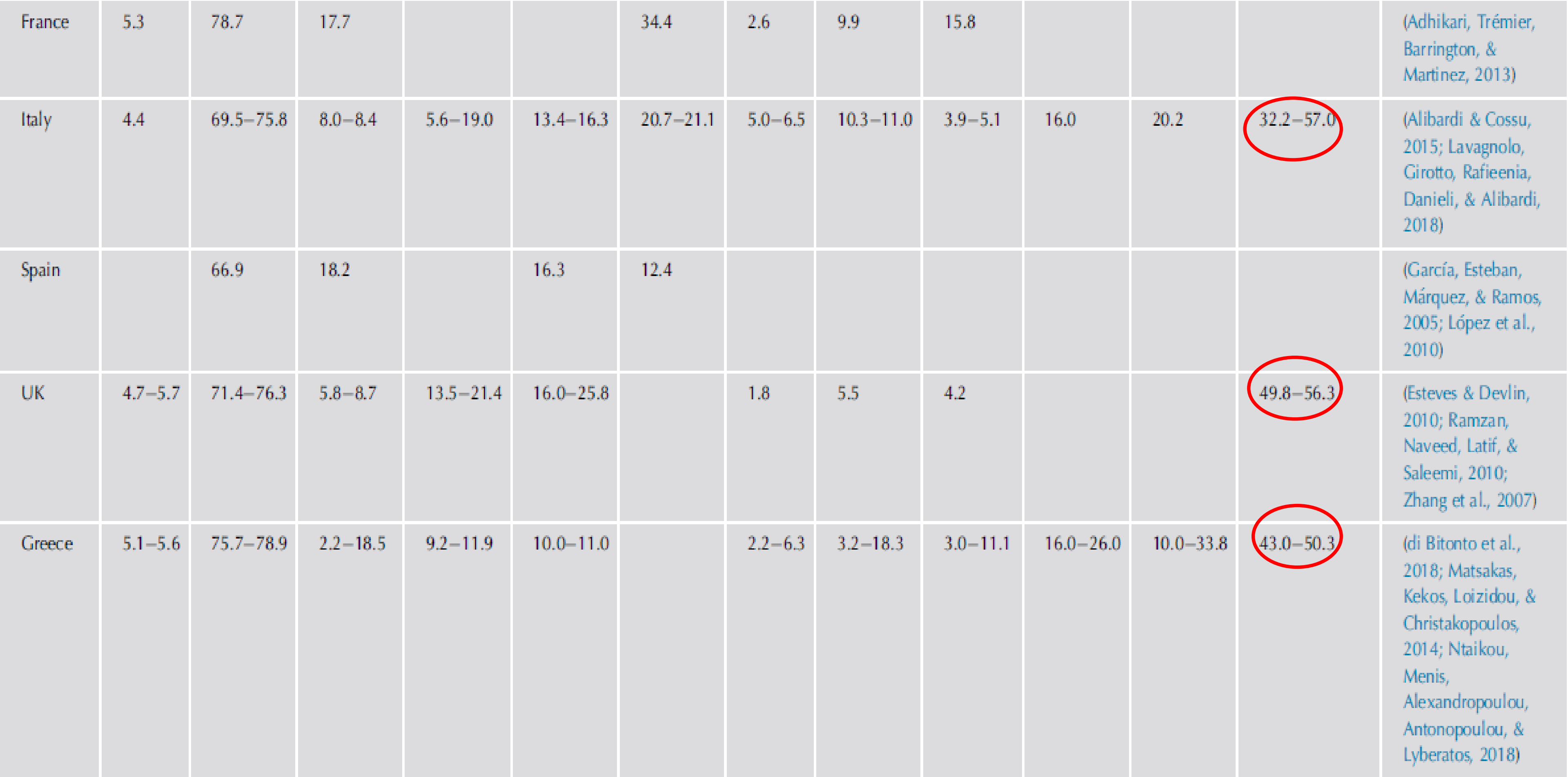


# Nutritional characteristics of organic fraction of municipal solid waste. All values are in dry basis.



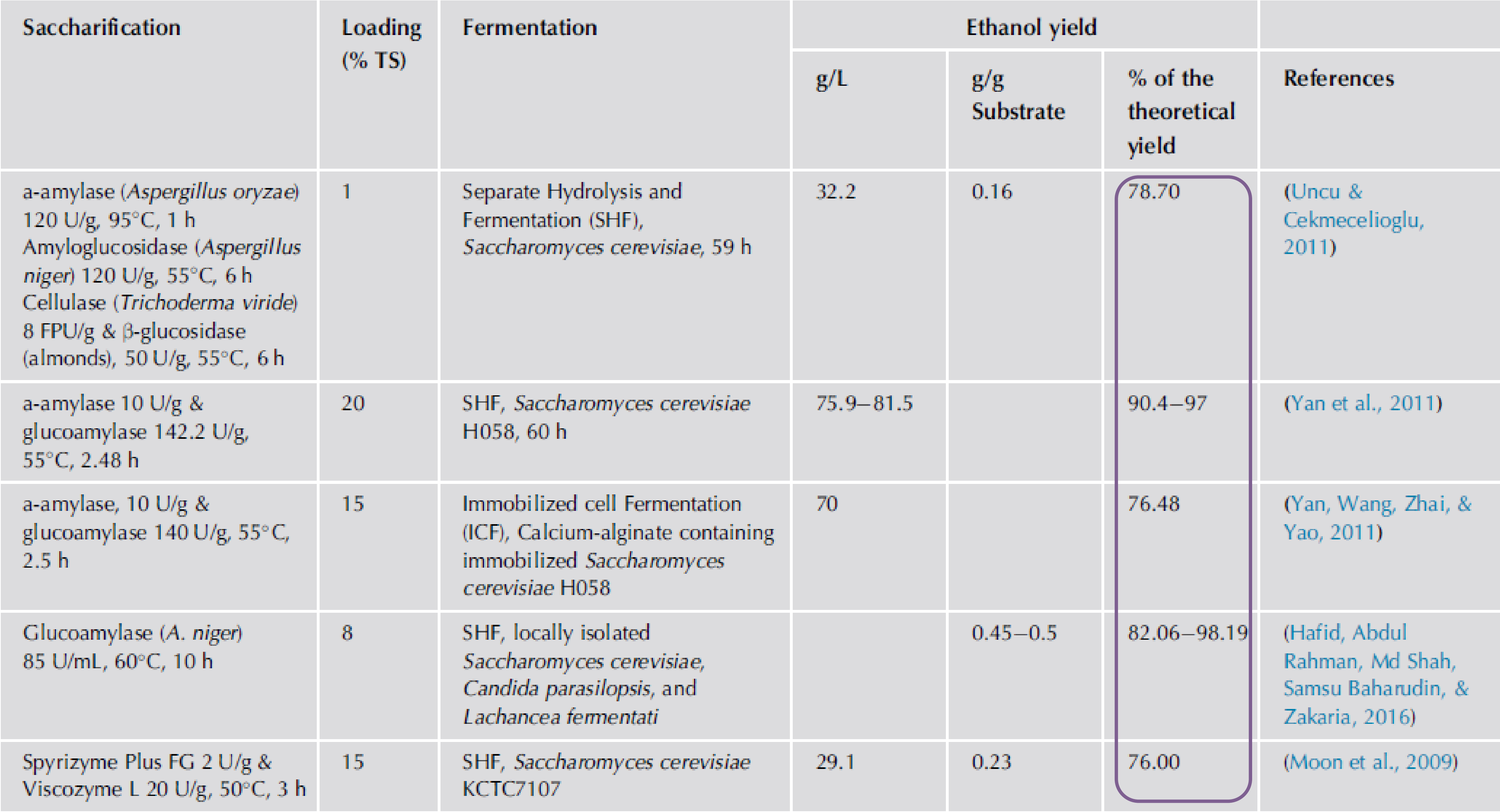
| | Country | pH | Moisture | Ash | Fat & oil | Protein | Raw fiber | Lignin | Cellulose | Hemi-cellulose | Starch | Free sugars | Total carbohydrates | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| America | USA | | 57.0–81.1 | 7.7 | 4.7–17.4 | 10.9–24.6 | 10.5–35.5 | | | | 6.7–25.9 | | | (Fung, Urriola, Baker, & Shurson, 2019) |
| Asia | Singapore | 4.0–4.5 | | 2.9 | 6.2–14.6 | 8.6–10.3 | | | 5.9 | | 60.3–62.7 | | 76.8 | (Ma, Cai, & Liu, 2017; Uçkun Kiran & Liu, 2015) |
| | China | 5.6 | 82.8 | | 18.1 | 15.6 | | | 2.3 | | 46.1 | 9.0 | | (Ma, Wang, Zhang, Xu, & Zou, 2008) |
| | India | | 85.0 | 11.5 | 8.5 | 6.8 | 33.5 | 8.5 | 15.5 | 9.5 | | | | (Rao & Singh, 2004) |
| | Turkey | | 64.4 | 5.1 | 24.7 | 12.6 | | | | | | | 60.0 | (Uncu & Cekmecelioglu, 2011) |
| Europe | Denmark | 5.3 | 64.4–72.0 | 14.4–20.0 | 8.1–16.6 | 8.1–16.6 | 3.5–31.5 | 15.9 | 4.3 | 11.3 | 11.7–17.0 | 4.9–9.0 | | (Davidsson, Gruvberger, Christensen, Hansen, & Jansen, 2007; La Cour Jansen, Spliid, Hansen, Svärd, & Christensen, 2004; López, Soliva, Martínez-Farré, Fernández, & Huerta-Pujol, 2010) |

# Nutritional characteristics of organic fraction of municipal solid waste. All values are in dry basis.



| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| France | 5.3 | 78.7 | 17.7 | | | 34.4 | 2.6 | 9.9 | 15.8 | | | | (Adhikari, Trémier, Barrington, & Martinez, 2013) |
| Italy | 4.4 | 69.5–75.8 | 8.0–8.4 | 5.6–19.0 | 13.4–16.3 | 20.7–21.1 | 5.0–6.5 | 10.3–11.0 | 3.9–5.1 | 16.0 | 20.2 | 32.2–57.0 | (Alibardi & Cossu, 2015; Lavagnolo, Girotto, Rafieenia, Danieli, & Alibardi, 2018) |
| Spain | | 66.9 | 18.2 | | 16.3 | 12.4 | | | | | | | (García, Esteban, Márquez, & Ramos, 2005; López et al., 2010) |
| UK | 4.7–5.7 | 71.4–76.3 | 5.8–8.7 | 13.5–21.4 | 16.0–25.8 | | 1.8 | 5.5 | 4.2 | | | 49.8–56.3 | (Esteves & Devlin, 2010; Ramzan, Naveed, Latif, & Saleemi, 2010; Zhang et al., 2007) |
| Greece | 5.1–5.6 | 75.7–78.9 | 2.2–18.5 | 9.2–11.9 | 10.0–11.0 | | 2.2–6.3 | 3.2–18.3 | 3.0–11.1 | 16.0–26.0 | 10.0–33.8 | 43.0–50.3 | (di Bitonto et al., 2018; Matsakas, Kekos, Loizidou, & Christakopoulos, 2014; Ntaikou, Menis, Alexandropoulou, Antonopoulou, & Lyberatos, 2018) |

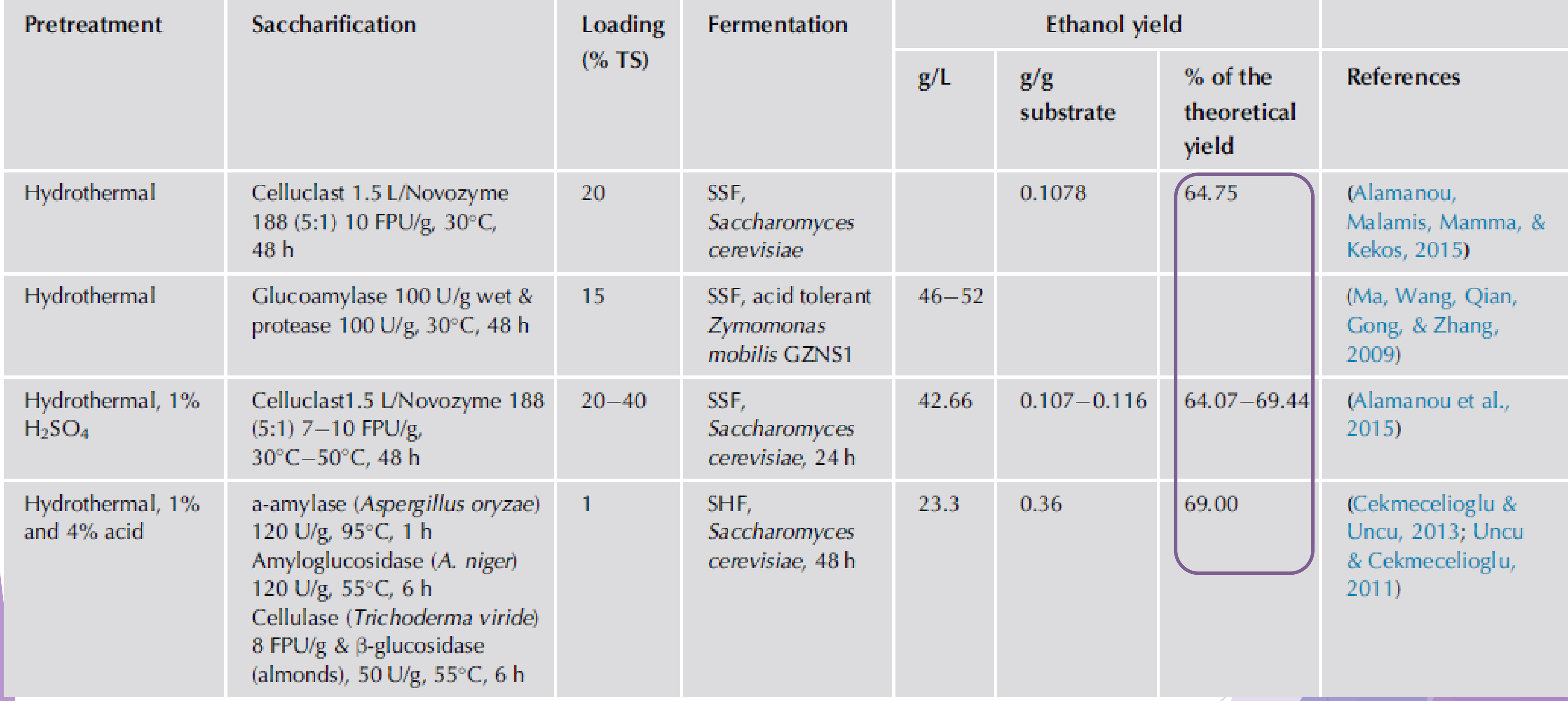
# Operational conditions



| Saccharification | Loading (% TS) | Fermentation | Ethanol yield | | | |
|---|---|---|---|---|---|---|
| | | | g/L | g/g Substrate | % of the theoretical yield | References |
| a-amylase (*Aspergillus oryzae*) 120 U/g, 95°C, 1 h Amyloglucosidase (*Aspergillus niger*) 120 U/g, 55°C, 6 h Cellulase (*Trichoderma viride*) 8 FPU/g & β-glucosidase (almonds), 50 U/g, 55°C, 6 h | 1 | Separate Hydrolysis and Fermentation (SHF), *Saccharomyces cerevisiae*, 59 h | 32.2 | 0.16 | 78.70 | (Uncu & Cekmecelioglu, 2011) |
| a-amylase 10 U/g & glucoamylase 142.2 U/g, 55°C, 2.48 h | 20 | SHF, *Saccharomyces cerevisiae* H058, 60 h | 75.9–81.5 | | 90.4–97 | (Yan et al., 2011) |
| a-amylase, 10 U/g & glucoamylase 140 U/g, 55°C, 2.5 h | 15 | Immobilized cell Fermentation (ICF), Calcium-alginate containing immobilized *Saccharomyces cerevisiae* H058 | 70 | | 76.48 | (Yan, Wang, Zhai, & Yao, 2011) |
| Glucoamylase (*A. niger*) 85 U/mL, 60°C, 10 h | 8 | SHF, locally isolated *Saccharomyces cerevisiae*, *Candida parasilopsis*, and *Lachancea fermentati* | | 0.45–0.5 | 82.06–98.19 | (Hafid, Abdul Rahman, Md Shah, Samsu Baharudin, & Zakaria, 2016) |
| Spyrizyme Plus FG 2 U/g & Viscozyme L 20 U/g, 50°C, 3 h | 15 | SHF, *Saccharomyces cerevisiae* KCTC7107 | 29.1 | 0.23 | 76.00 | (Moon et al., 2009) |

Total solids

# Operational conditions

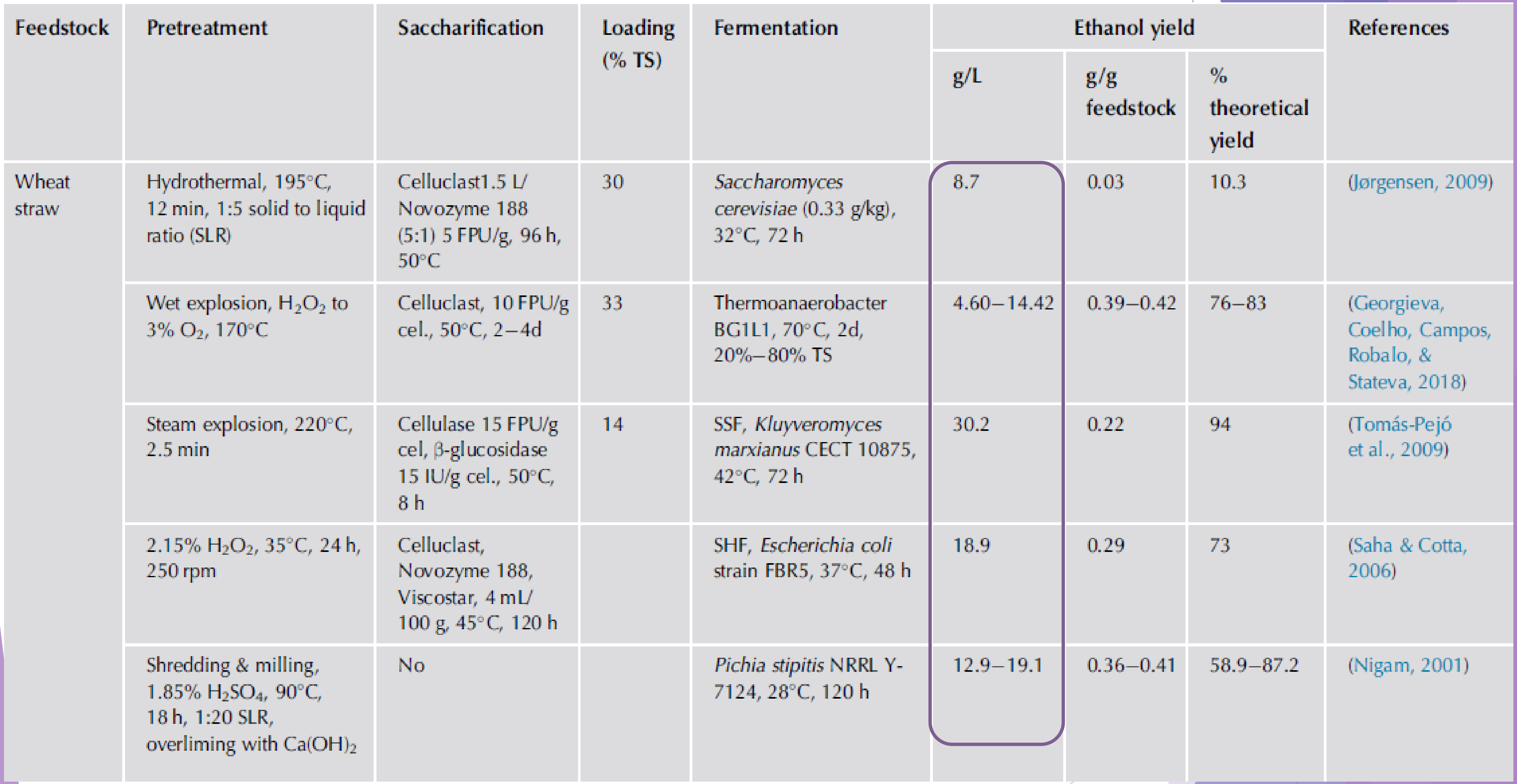


| Pretreatment | Saccharification | Loading (% TS) | Fermentation | Ethanol yield | | | References |
|---|---|---|---|---|---|---|---|
| | | | | g/L | g/g substrate | % of the theoretical yield | |
| Hydrothermal | Celluclast 1.5 L/Novozyme 188 (5:1) 10 FPU/g, 30°C, 48 h | 20 | SSF, *Saccharomyces cerevisiae* | | 0.1078 | 64.75 | (Alamanou, Malamis, Mamma, & Kekos, 2015) |
| Hydrothermal | Glucoamylase 100 U/g wet & protease 100 U/g, 30°C, 48 h | 15 | SSF, acid tolerant *Zymomonas mobilis* GZNS1 | 46–52 | | | (Ma, Wang, Qian, Gong, & Zhang, 2009) |
| Hydrothermal, 1% $H_2SO_4$ | Celluclast1.5 L/Novozyme 188 (5:1) 7–10 FPU/g, 30°C–50°C, 48 h | 20–40 | SSF, *Saccharomyces cerevisiae*, 24 h | 42.66 | 0.107–0.116 | 64.07–69.44 | (Alamanou et al., 2015) |
| Hydrothermal, 1% and 4% acid | a-amylase (*Aspergillus oryzae*) 120 U/g, 95°C, 1 h<br>Amyloglucosidase (*A. niger*) 120 U/g, 55°C, 6 h<br>Cellulase (*Trichoderma viride*) 8 FPU/g & β-glucosidase (almonds), 50 U/g, 55°C, 6 h | 1 | SHF, *Saccharomyces cerevisiae*, 48 h | 23.3 | 0.36 | 69.00 | (Cekmecelioglu & Uncu, 2013; Uncu & Cekmecelioglu, 2011) |

# Composition of agricultural residues

| Agricultural residues | Cellulose | Hemicellulose | Lignin | Crude protein | Ash |
|---|---|---|---|---|---|
| Wheat straw | 30.2–55.6 | 4.2–30.0 | 7.9–27.5 | 4.4 | 4–4.7 |
| Corn straw | 31.8–49.3 | 19.6–33.4 | 7.0–17.6 |  | 4.2–12.5 |
| Corn stalks | 10.8–39.6 | 10.7–60.3 | 2.0–27.9 |  | 2.0–8.5 |
| Corn stover | 27.0–39.6 | 15.7–32.5 | 4.6–18.4 | 0.7–9.3 | 4.1–7.2 |
| Corn cobs | 33.7–44.0 | 31.0–49.6 | 6.1–18.0 | 4.3 | 2.9–3.2 |
| Rice straw | 29.2–38.9 | 18.7–25.9 | 13.3–22.1 |  | 11.3–17.3 |
| Sugarcane straw | 32.4–33.6 | 21.7–25.5 | 31.8–36.3 |  | 5.7–6.0 |
| Soybean straw | 25.0–74.0 | 10.3–56.0 | 5.0–21.6 |  | 0–5.2 |
| Barley straw | 33.8–44.0 | 16.9–33.0 | 13.8–20.7 |  | 2.5–3.9 |
| Cotton stalks | 14.4–43.7 | 12.5–23.9 | 21.5–29.4 |  | 4.8 |

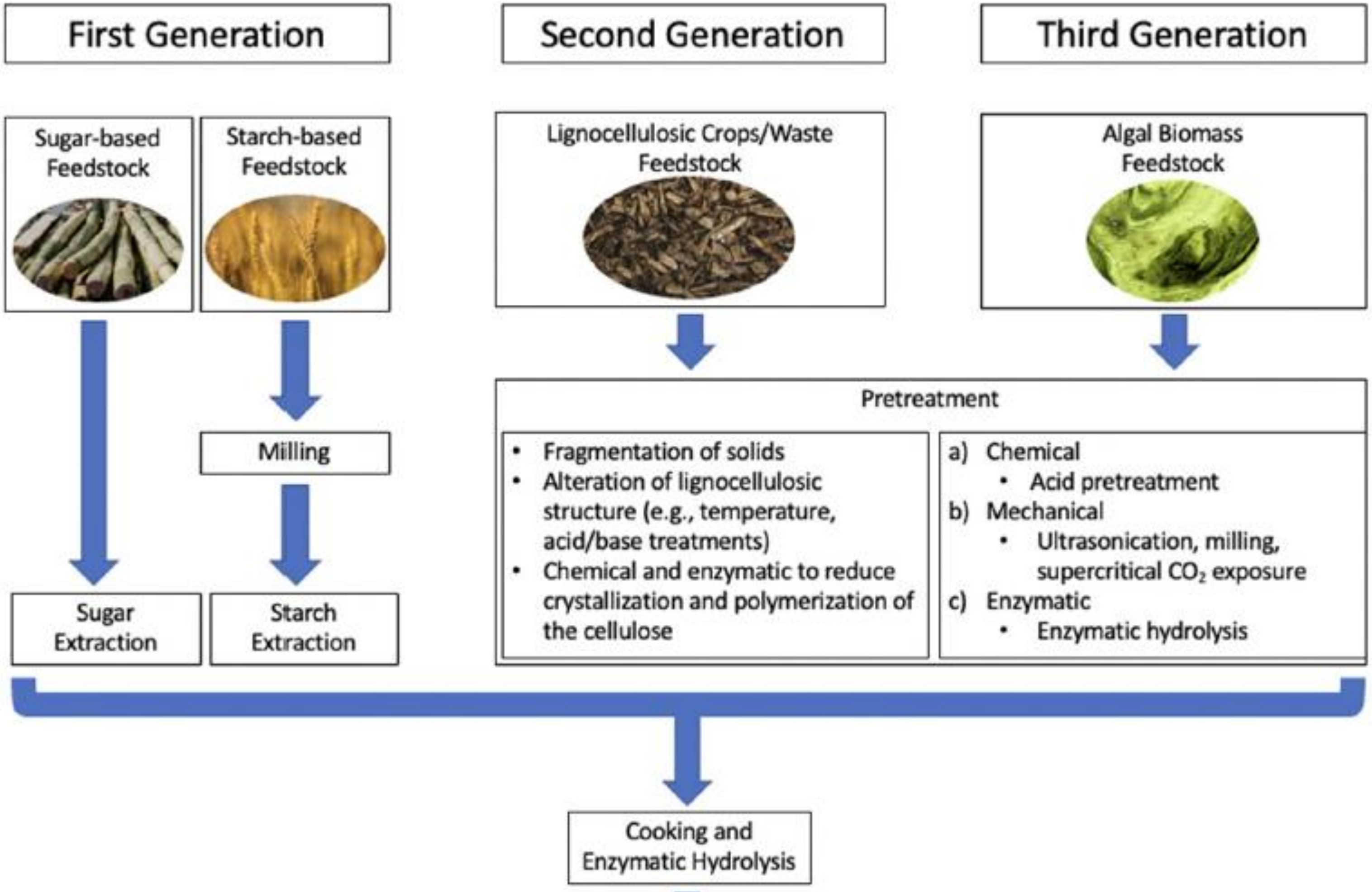
# Ethanol from agricultural residues



| Feedstock | Pretreatment | Saccharification | Loading (% TS) | Fermentation | Ethanol yield | | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | | g/L | g/g feedstock | % theoretical yield | |
| Wheat straw | Hydrothermal, 195°C, 12 min, 1:5 solid to liquid ratio (SLR) | Celluclast1.5 L/ Novozyme 188 (5:1) 5 FPU/g, 96 h, 50°C | 30 | *Saccharomyces cerevisiae* (0.33 g/kg), 32°C, 72 h | 8.7 | 0.03 | 10.3 | (Jørgensen, 2009) |
| | Wet explosion, $H_2O_2$ to 3% $O_2$, 170°C | Celluclast, 10 FPU/g cel., 50°C, 2–4d | 33 | Thermoanaerobacter BG1L1, 70°C, 2d, 20%–80% TS | 4.60–14.42 | 0.39–0.42 | 76–83 | (Georgieva, Coelho, Campos, Robalo, & Stateva, 2018) |
| | Steam explosion, 220°C, 2.5 min | Cellulase 15 FPU/g cel, β-glucosidase 15 IU/g cel., 50°C, 8 h | 14 | SSF, *Kluyveromyces marxianus* CECT 10875, 42°C, 72 h | 30.2 | 0.22 | 94 | (Tomás-Pejó et al., 2009) |
| | 2.15% $H_2O_2$, 35°C, 24 h, 250 rpm | Celluclast, Novozyme 188, Viscostar, 4 mL/ 100 g, 45°C, 120 h | | SHF, *Escherichia coli* strain FBR5, 37°C, 48 h | 18.9 | 0.29 | 73 | (Saha & Cotta, 2006) |
| | Shredding & milling, 1.85% $H_2SO_4$, 90°C, 18 h, 1:20 SLR, overliming with $Ca(OH)_2$ | No | | *Pichia stipitis* NRRL Y-7124, 28°C, 120 h | 12.9–19.1 | 0.36–0.41 | 58.9–87.2 | (Nigam, 2001) |

Municipal waste can be as valuable source as agricultural residues

# Review of potential sources



**First Generation**

**Second Generation**

**Third Generation**

Sugar-based Feedstock

Starch-based Feedstock

Lignocellulosic Crops/Waste Feedstock

Algal Biomass Feedstock

Milling

Sugar Extraction

Starch Extraction

Pretreatment

- Fragmentation of solids
- Alteration of lignocellulosic structure (e.g., temperature, acid/base treatments)
- Chemical and enzymatic to reduce crystallization and polymerization of the cellulose

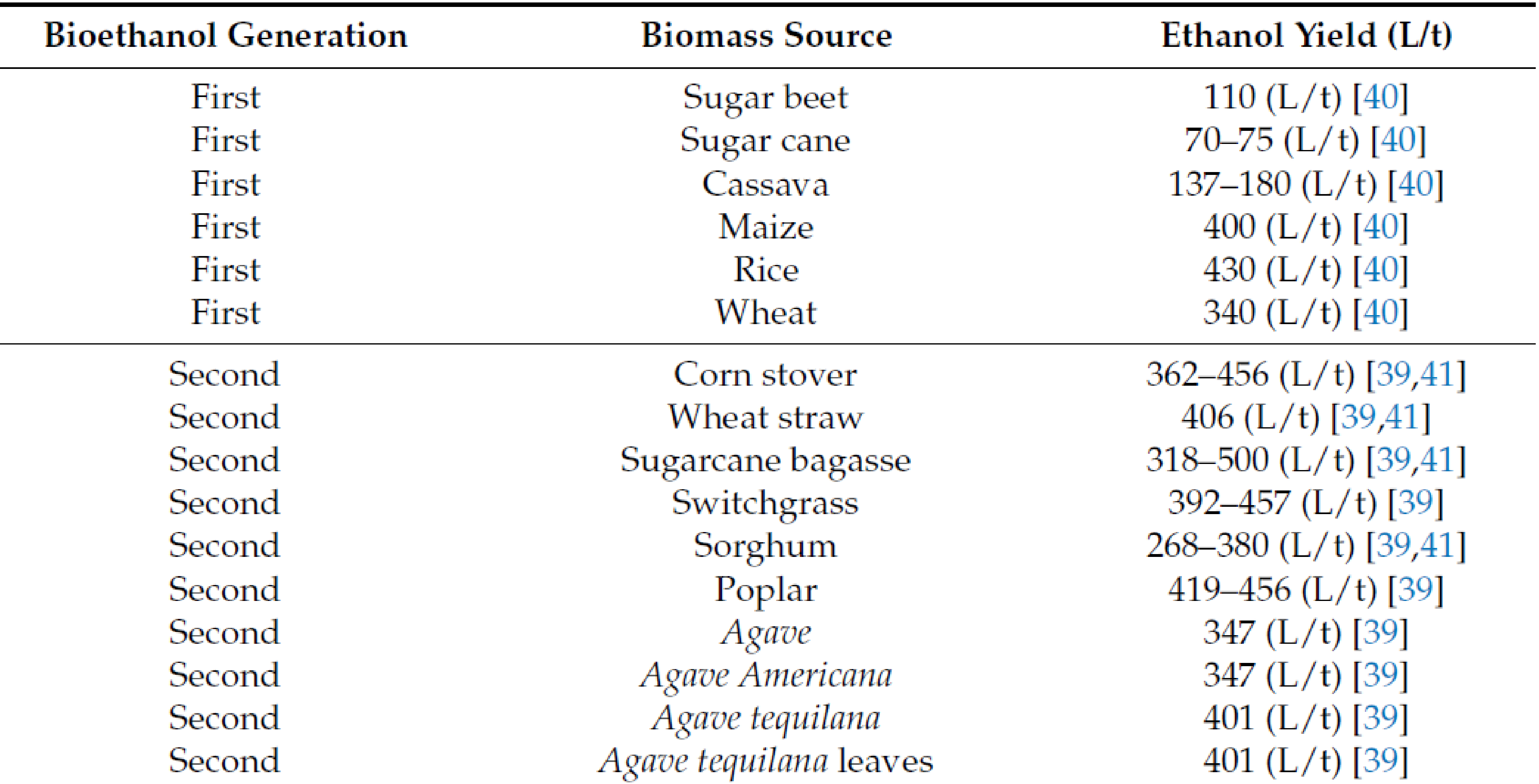
a) Chemical
  - Acid pretreatment

b) Mechanical
  - Ultrasonication, milling, supercritical $CO_2$ exposure

c) Enzymatic
  - Enzymatic hydrolysis

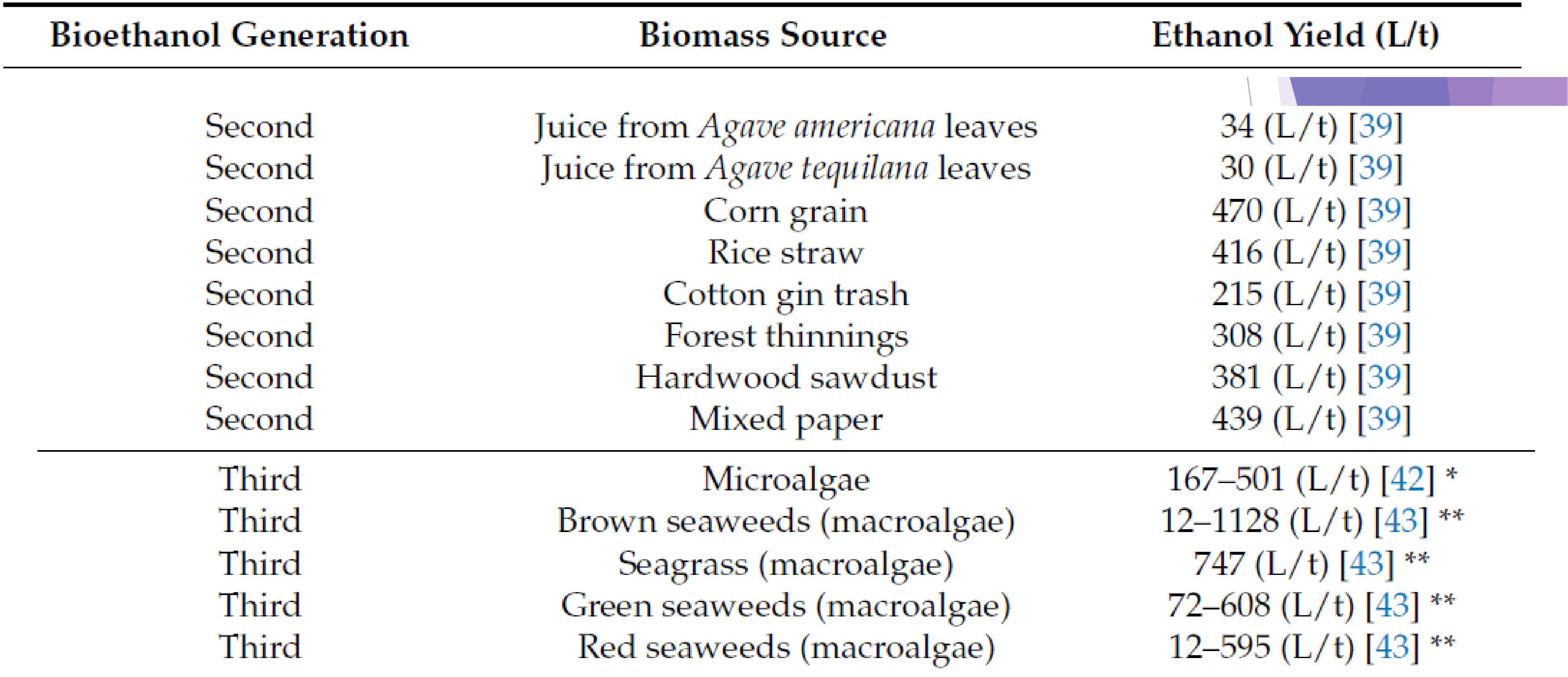
Cooking and Enzymatic Hydrolysis

# Bioethanol yields



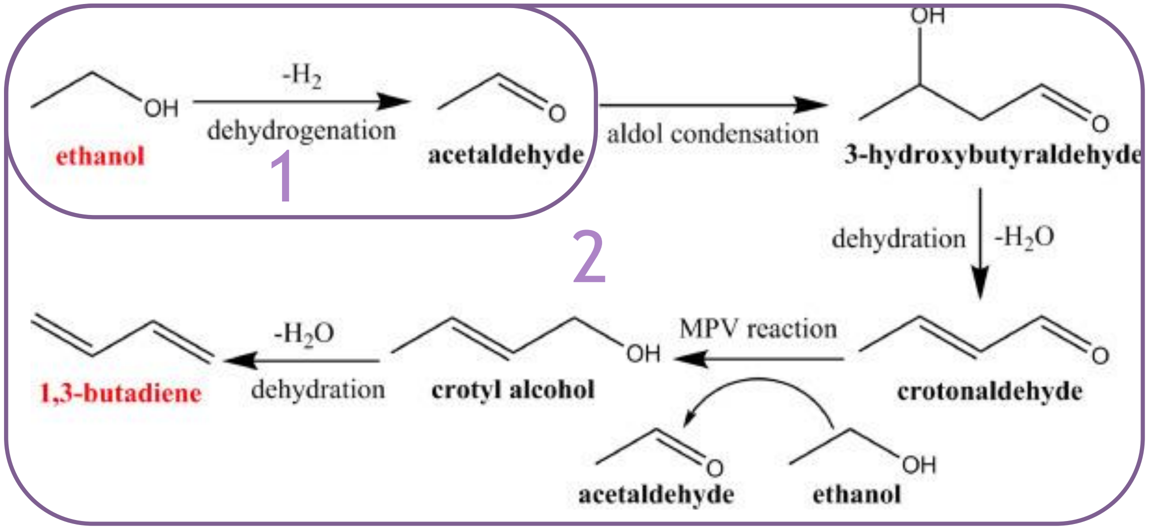
| Bioethanol Generation | Biomass Source | Ethanol Yield (L/t) |
|---|---|---|
| First | Sugar beet | 110 (L/t) [40] |
| First | Sugar cane | 70–75 (L/t) [40] |
| First | Cassava | 137–180 (L/t) [40] |
| First | Maize | 400 (L/t) [40] |
| First | Rice | 430 (L/t) [40] |
| First | Wheat | 340 (L/t) [40] |
| Second | Corn stover | 362–456 (L/t) [39,41] |
| Second | Wheat straw | 406 (L/t) [39,41] |
| Second | Sugarcane bagasse | 318–500 (L/t) [39,41] |
| Second | Switchgrass | 392–457 (L/t) [39] |
| Second | Sorghum | 268–380 (L/t) [39,41] |
| Second | Poplar | 419–456 (L/t) [39] |
| Second | *Agave* | 347 (L/t) [39] |
| Second | *Agave Americana* | 347 (L/t) [39] |
| Second | *Agave tequilana* | 401 (L/t) [39] |
| Second | *Agave tequilana* leaves | 401 (L/t) [39] |

# Bioethanol yields



| Bioethanol Generation | Biomass Source | Ethanol Yield (L/t) |
|---|---|---|
| Second | Juice from *Agave americana* leaves | 34 (L/t) [39] |
| Second | Juice from *Agave tequilana* leaves | 30 (L/t) [39] |
| Second | Corn grain | 470 (L/t) [39] |
| Second | Rice straw | 416 (L/t) [39] |
| Second | Cotton gin trash | 215 (L/t) [39] |
| Second | Forest thinnings | 308 (L/t) [39] |
| Second | Hardwood sawdust | 381 (L/t) [39] |
| Second | Mixed paper | 439 (L/t) [39] |
| Third | Microalgae | 167–501 (L/t) [42] * |
| Third | Brown seaweeds (macroalgae) | 12–1128 (L/t) [43] ** |
| Third | Seagrass (macroalgae) | 747 (L/t) [43] ** |
| Third | Green seaweeds (macroalgae) | 72–608 (L/t) [43] ** |
| Third | Red seaweeds (macroalgae) | 12–595 (L/t) [43] ** |

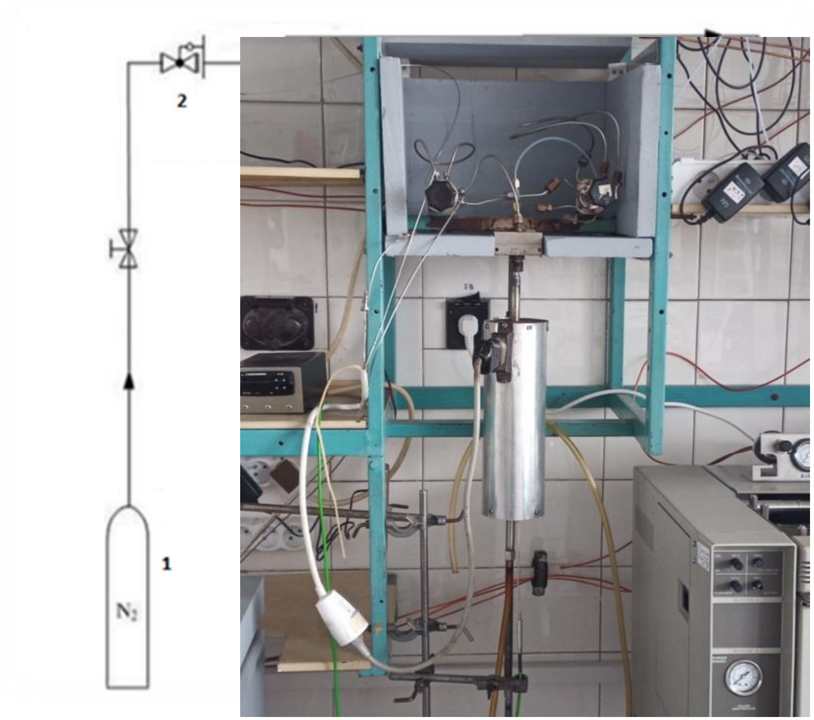
# Multistep reaction scheme, both redox and acidobasically catalysed



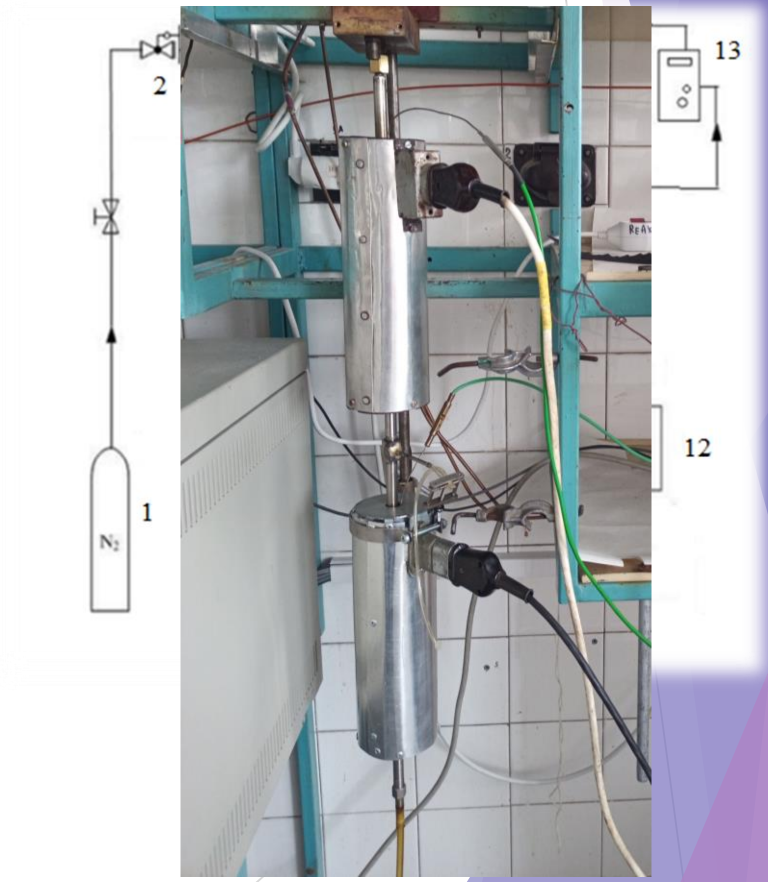
1 – mainly redox catalysed
2- acidobasic catalysis

# One-step vs. Two-step process

One-step

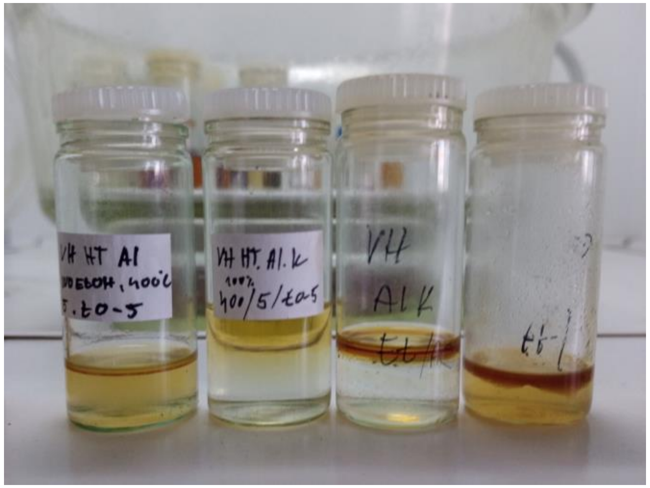


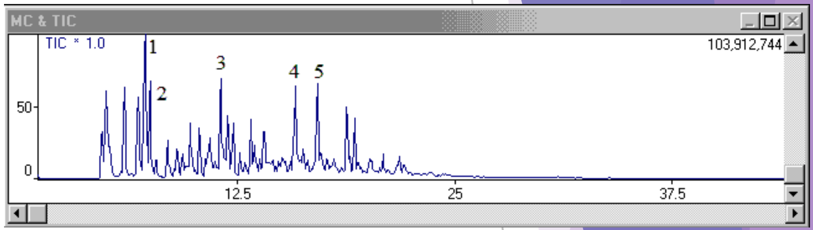
Two-steps



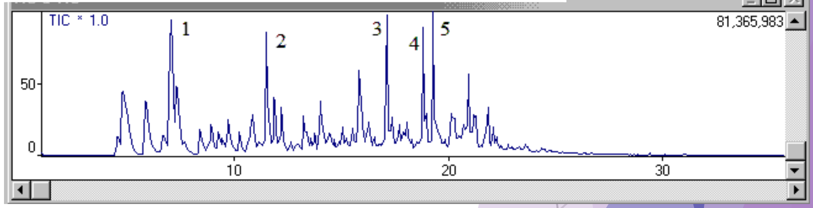
# Mg-Al (excess Al) hydrotalcite, liquid products

- 1-step with ethanol-acetaldehyde raw
- 2-step with ethanol only





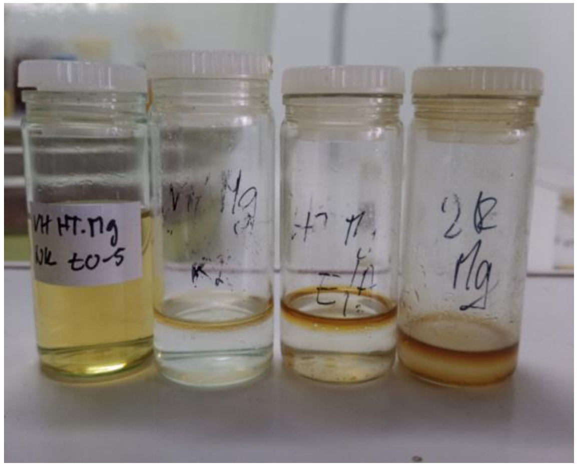
| | |
|---|---|
| 1 | **2-Butenal** |
| 2 | Bicyclo (3,3,1)nonan-2-one |
| 3 | 3-Octyn-2-ol |
| 4 | Acetophenone |
| 5 | 2-Methyl-1-Propenyl-Benzene |

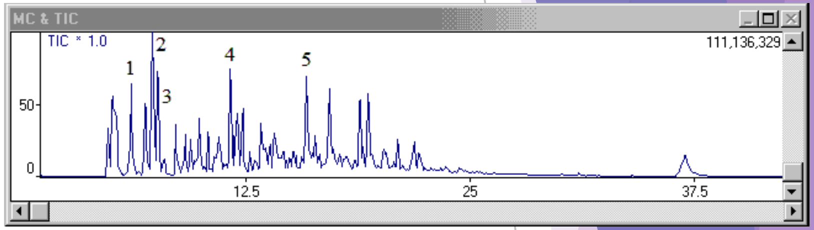


| | |
|---|---|
| 1 | **1-Butanol** |
| 2 | Izobutyl Izobutyrate |
| 3 | 2,3-Dimethyl-Phenol |
| 4 | 2-Ethyl-6-Methyl-Phenol |
| 5 | 2-Ethyl-4,5-Dimethyl-Phenol |

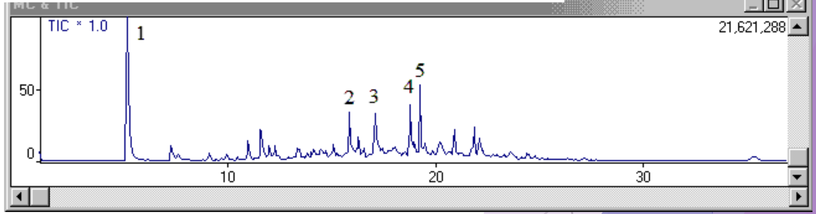
# Mg-Al (excess Mg) hydrotalcite, liquid products

- 1-step with ethanol-acetaldehyde raw
- 2-step with ethanol only



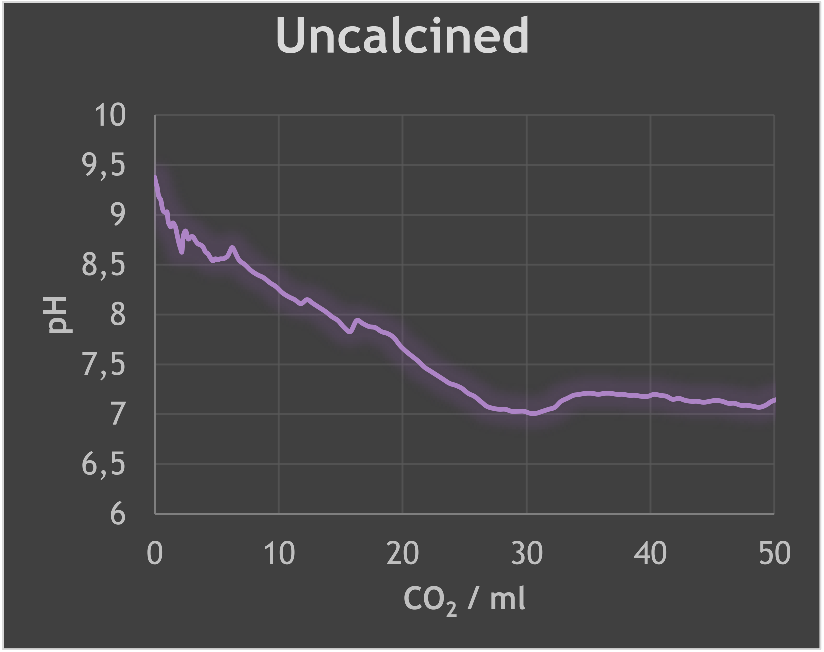


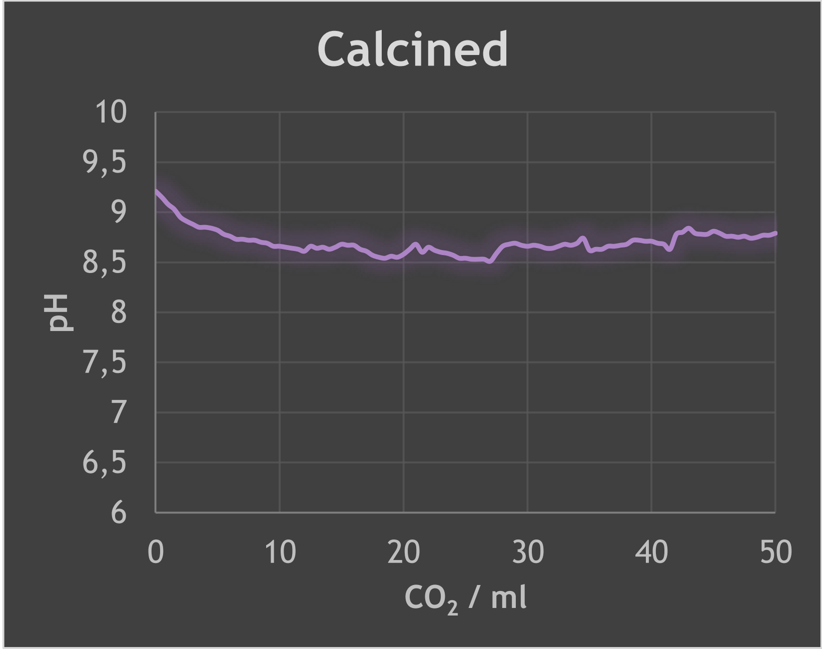
| | |
|---|---|
| 1 | Butanal |
| 2 | 1-Butanol |
| 3 | 3-Methyl-3-buten-2-ol |
| 4 | 2,5-Dimethyl-1,5-Hexadien-3-ol |
| 5 | 3-Methyl-1-Phenyl-2-Butanone |



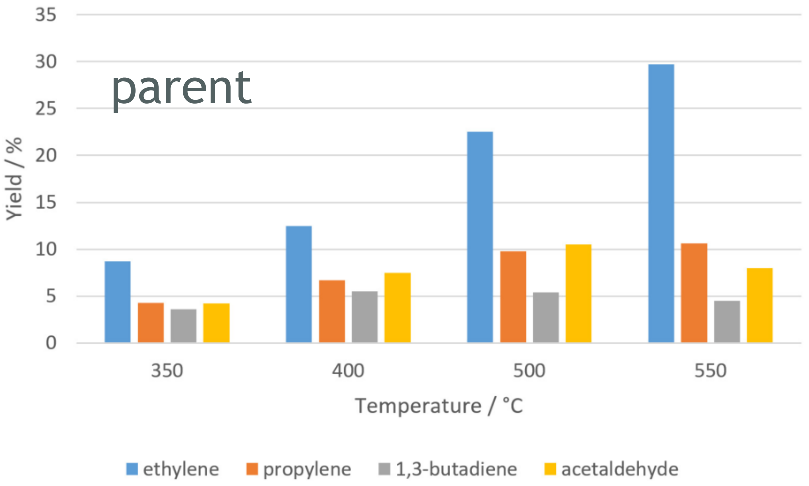
| | |
|---|---|
| 1 | Acetone |
| 2 | Nonan-4-one |
| 3 | 3-Ethyl-Phenol |
| 4 | 2-Ethyl-6-Methyl-Phenol |
| 5 | 2-Ethyl-4,6-Dimethyl-Phenol |

# Estimation of the acidity
# Titration with aqueous solution of $CO_2$

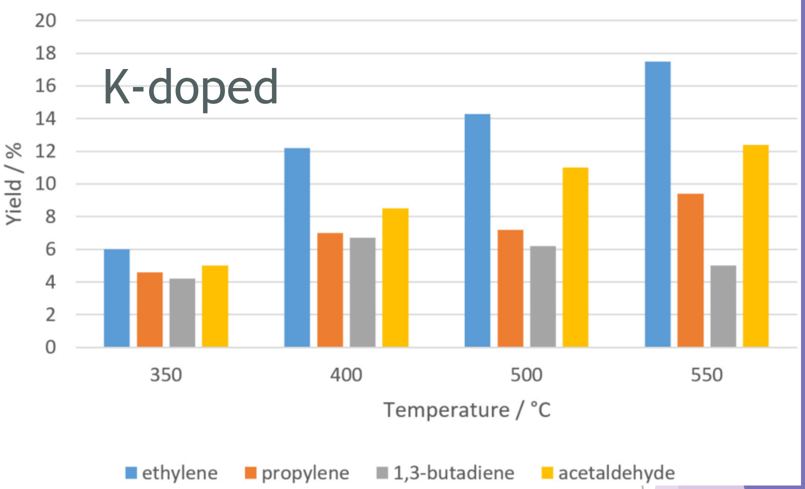




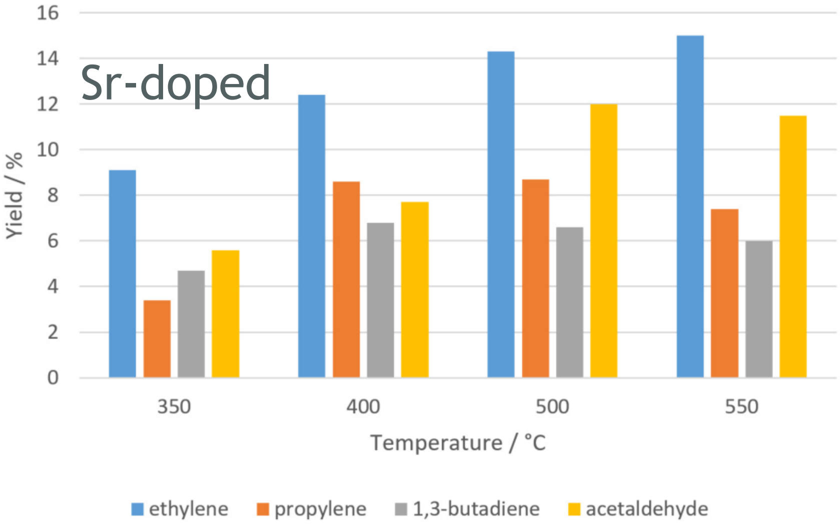
# Basic magnesium silicate: $Mg_4Si_6O_{15}(OH)_2 \cdot 6H_2O$ - white clay mineral occurring in the nature



parent



K-doped

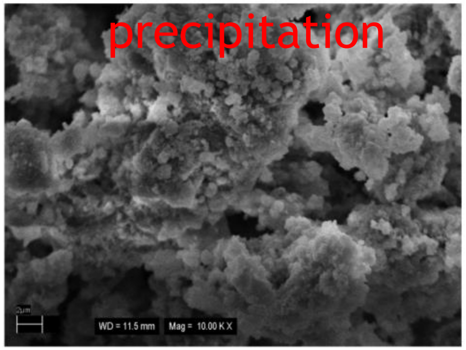


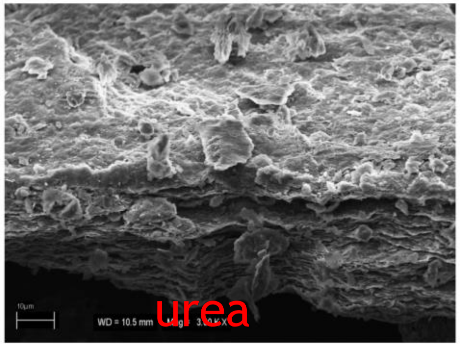
Sr-doped

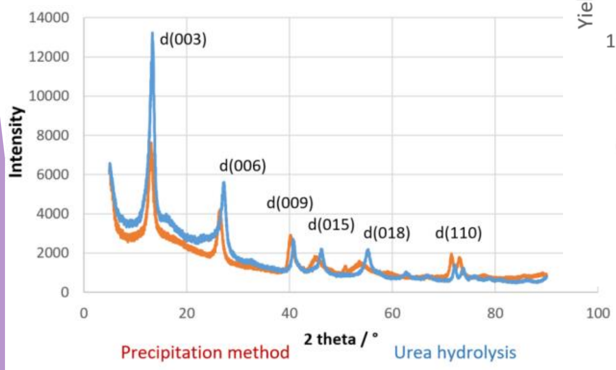
- The parent sepiolite is not expected to be acidic
- However, addition of strong bases improves the butadiene/ethylene ratio

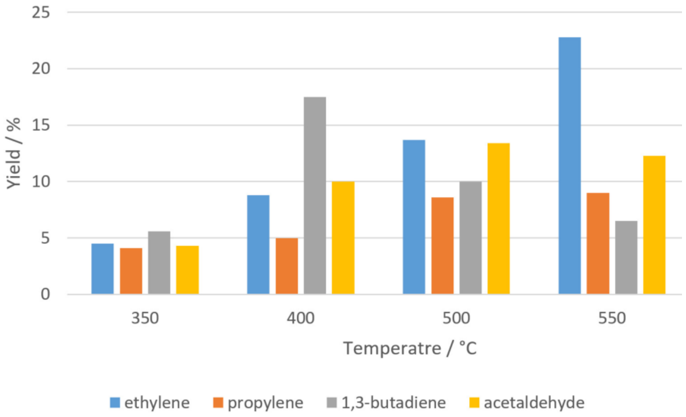
# Si-Al instead of Si-Mg

A layered double hydroxide: $Mg_6Al_2CO_3(OH)_{16}\cdot 4H_2O$ –hydrotalcite. Depending on the particle size, its water suspension has a pH 7-9

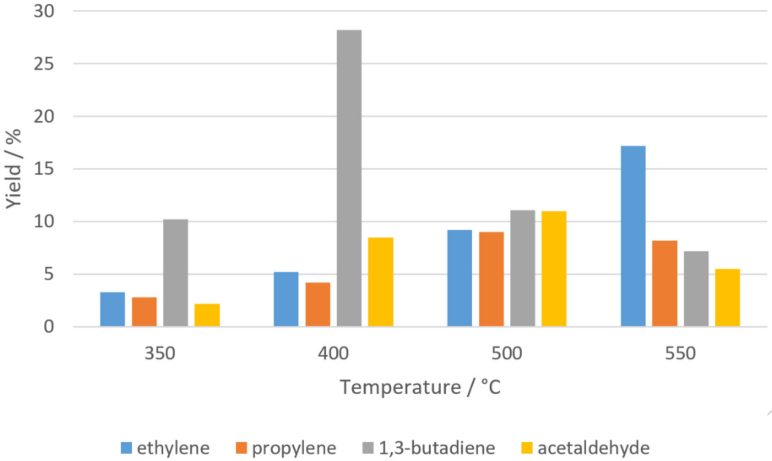








- **Mg and Al nitrates precipitated by NaOH + $Na_2CO_3$**



- **Mg and Al nitrates precipitated by urea hydrolysis at 100 °C**

# Conclusions

- **The microstructure of the catalyst has a significant effect on the product distribution**
- **Suppressing the acidity of the catalyst (by K, Sr) leads to a more favorable ethylene/butadiene ratio**
- **It is possible to estimate the basicity of the catalyst by facile $CO_2$ – titration**
- **The ethanol conversion can be led in a single-bed or double-bed reactor design**
- **The formation of phenolic compounds was observed, depending on the reactor design**
- **The lignocellulosic and other third-generation sources (as algae) of fermentable sugars are still underestimated**

# Thank you for your attention!

## Acknowledgement

**European Regional Development Fund (Interreg, SKHU/1902/4.1/001/Bioeconomy)**
**www.skhu.eu**

Interreg
Slovakia-Hungary

Building Partnership